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INVESTIGATION OF THE POSSIBILITY OF ORAL IMMUNIZATION OF EXPERIMENTAL ANIMALS WITH THE SMALLPOX VACCINE VIRUS

Report I.

Immunological Changes in the Vaccinated Animals

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High contagiousness and gravity of the course of natural smallpox require in cases of epidemic outbreaks a mass immunization of the population within the shortest period of time. The epicutaneous vaccination which on the whole meets the requirements of the mass vaccination method, nevertheless envisages the need of a large contingent of qualified personnel, organization of special points, etc. Besides, the vaccine is not always safe, especially in those vaccinated for the first time, and may induce a strong general reaction.

The advances of peroral immunization of humans with live vaccines against tuberculosis (Botez and coauthors, 1963, et al.) and poliomyelitis, as well as the positive results obtained experimentally with a peroral immunization with live vaccines against brucellesis (Korolev and Konstant, 1960) tularemia (Mikhaylov, 1952; El'bert, 1952), Q fever (Borob'yev and Pautov, 1964), Newcastle chicken disease (Marek and Raszewska, 1958; Kolosov and Syurin, 1964) and some other infections, prompted us to investigate the peroral immunization with smallpox vaccine.

We found in literature only two references in regard to this possibility. In 1934, Felix elicited cutaneous immunity in rabbits after intragastric administration of the smallpox

vaccine in capsules, or directly in the small intestine following laparotomy (cited from Morozov, 1938). In the monograph of Solov'yev and Mastyukova (1961) there is also a reference to the development of vaccine immunity following oral administration of the virus (p 231).

A definite drawback of peroral immunization with smallpox vaccine is the acid gastric content because, according to the data of Beard and coauthors (1935), at pH under 2.5 the virus becomes immediately inactivated. However, there was no reason to deny the possibility of penetration of the virus through the mucosa of the upper part of the gastrointestinal tract, as well as the passing of some viable viral particles through the stomach into the small intestine (Konn, 1958), where the conditions are favorable to the virus.

Experiments were conducted on guinea pigs, rabbits and monkeys. The animals received perorally (or through a gastric tube directly in the stomach) the virus material in the form of a suspension of infected chorioallantoic membranes of 12-14-day-old chick embryos. Prior to immunization and within 3-4 weeks after it, the antihemagglutinins and virus-neutralizing antibodies were determined in the serum; in rabbits, in addition, the cutaneous immunity was investigated via intracutaneous inoculation of the smallpox vaccine, as per Grott. The viral activity was determined by titration on tissue cultures (fibroblasts of chick embryos), or on the developed chick embryos.

In various experiments the rabbits received from $5 \cdot 10^2$ to $1.5 \cdot 10^7$ variola-forming units (OU, upon titration on chick embryos), cytopathic doses ($TTs^{50}D_{50}$ [titrirovannaya tsitopaticheskaya doza 50; titrated cytopathic dose 50] upon titration on tissue cultures) of the virus of the smallpox vaccine (strain No 3 of the dermovaccine of the Institute of Embryology and Microbiology im. Gamaleya, adapted to chick embryos). We observed no substantial differences in the titer of antihemagglutinins and in skin immunity, upon comparison according to the X criterion (Ashmarin and Vorob'yev, 1962). The only differences observed were in the frequency of positive responses to immunization (Table 1).

Upon 2- and 3-fold internal administration of smallpox vaccine, the antihemagglutinin titers and strength of cutaneous immunity as checked by the Grott method also showed no substantial difference from the results of one-stage immunization.

Guinea pigs turned out to be less sensitive to the smallpox vaccine virus following peroral immunization, and the titer value of hemagglutinins was lower than in the immunization of rabbits (Table 2).

Table 1.

Determination of Minimal Immunizing Dose of Smallpox Vaccine, Following Peroral Administration to Rabbits

(1) Доза вируса (в $ТТсПД_{50}$)	(2) Число кроликов	(3) Средний титр антигемагглютининов по группам	(4) Сумма диаметров вакцинальных инфильтратов (в см) при заражении по Гроту (среднее по группам)	(5) Примечания
$5 \cdot 10^7$	6	1:160	1,3	(7) У всех кроликов титры антигемагглютининов 1:80 и выше
$5 \cdot 10^6$	6	1:160	1,2	(8) У 1 кролика титр антигемагглютининов 1:20, у остальных 1:80
$5 \cdot 10^5$	6	1:160	1,4	(9) У 1 кролика титр антигемагглютининов 1:10, у остальных 1:80 и выше
$5 \cdot 10^4$	6	<1:10	2,4	(10) У 1 кролика титр антигемагглютининов 1:80, у одного 1:10, у остальных <1:10
$5 \cdot 10^3$	5	<1:10	2,9	(11) У всех кроликов титр антигемагглютининов <1:10
(6) Контроль	5	<1:10	2,5	

1 -- Viral dose (in $TTsPD_{50}$); 2 -- Number of rabbits; 3 -- Mean titer of antihemagglutinins, according to groups; 4 -- Sum of diameters of vaccine infiltrates (in centimeters), upon inoculation according to Grott (average, as per groups); 5 -- Annotations; 6 -- Control; 7 -- In all rabbits the titers of antihemagglutinins are 1:80 and higher; 8 -- In one rabbit the antihemagglutinin titer was 1:20, in the rest -- 1:80; 9 -- In one rabbit the antihemagglutinin titer was 1:10, in the rest 1:80 and higher; 10 -- In one rabbit the hemagglutinin titer was 1:80, in one -- 1:10, in the rest <1:10; 11 -- In all rabbits the antihemagglutinin titer was <1:10.

It has been thus ascertained that rabbits and guinea pigs developed immunity following peroral administration of the smallpox virus. The rabbits responded by production of antihemagglutinins per dose equalling $5 \cdot 10^6$-$1 \cdot 10^8$ $TTsPD_{50}$, whereas in the immunization of guinea pigs a dose approximately 10-20-fold was required. No macro- and micro-scopic changes in mucosa of the mouth, pharynx, esophagus and small intestine were observed (histological examinations were made by A. M. Igonin).

In the immunization of monkeys, the virus-containing suspension was mixed with sugar and administered in the amount of

$5 \cdot 10^5$-$1 \cdot 10^6$ $TTsPD_{50}$. Following immunization of 21 monkeys, no macroscopic changes of oral mucosa were observed. The animals felt well. Prior to immunization, there were virtually no antihemagglutinins detected in the blood (<1:10), and virus-neutralizing antibodies were found only in a non-diluted serum in a few animals. Following immunization, the antihemagglutinins were detected in 1:10-1:160 titers (1:140 on the average), and virus-neutralizing antibodies -- up to 1:1000.

Table 2.

Immunological Response of Rabbits and Guinea Pigs to Peroral Administration of the Smallpox Vaccine Virus

(1) Вид животного	(4) Иммунологические сдвиги при введении внутрь разных доз оспенно-образующих единиц вируса			
	10^3	10^4	10^5	10^6
(2) Кролики	1/6	5/6	23/23	11/11
(3) Морские свинки . . .	0/10	2/25	9/30	13/15

Designations: numerator -- the number of animals with immune changes; denominator -- number of animals in the experiment.

1 -- Animal species; 2 -- Rabbit ; 3 -- Guinea pigs; 4 -- Immunological changes following internal administration of various doses of variola-forming virus units.

Thus, in experiments with three species of tested animals (guinea pigs, rabbits and monkeys) it was demonstrated that the smallpox vaccine introduced perorally is capable of producing immunity.

Conclusion

A live smallpox virus administered perorally to experimental animals (monkeys, rabbits and guinea pigs) induced immunological changes (a rise in the antihemagglutinin titers and virus-neutralizing antibodies). Simultaneously, there was an increase of cutaneous immunity, determined by intracutaneous inoculation of rabbits with the smallpox vaccine, as per Grott.

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